to support one over the others. We historically used CSA/MTX (n = 187 pts). Over the last 3 years we used CSA/MMF (n = 92). Median pt age was 52 years (18-73). Diagnoses included AML/MDS (n = 152), ALL (n = 30), CML (n = 12), myeloma (n = 24), lymphoma (n = 40), others (n = 21). The donor was 10/10 (n = 191), 9/10 (n = 54) or ≤ 8/10 match (n = 34). Conditioning was myeloablative (n = 58) or reduced intensity/toxicity (RIC, n = 221). Non-relapse mortality (NRM) is dependant on regimen toxicity and GVHD. CSA/MMF was less toxic than CSA/MTX. It allowed faster engraftment, day +11 and +14, respectively ($p < 0.001$). Day30 mortality was 2.2% and 10.7%, respectively ($p = 0.04$). Multivariate analysis (MVA) identified high comorbidity index (HR 2.5, $p = 0.05$), advanced disease (HR 6.2, $p = 0.005$), mismatched donor (HR 2.4, $p = 0.03$) and lymphoid malignancies (HR 2.8, $p = 0.03$) as adverse factors for regimen-related mortality. CSA/MMF was protective (HR 0.4, $p = 0.02$). However, CSA/MMF was less effective in preventing grade III-IV acute GVHD; cumulative incidence 29% and 18%, respectively ($p = 0.005$). MVA identified mismatched donor (HR 3.4, $p = 0.001$) and CSA/MMF (HR 2.4, $p = 0.004$) as adverse factors, while RIC was protective (HR 0.4, $p = 0.01$). The net effect was that NRM was equivalent with both regimens. GVHD regimen had major impact on overall survival (OS) when pts were stratified based on disease status. In early stage disease, OS was 65% and 42%, after CSA/MTX and CSA/MMF, respectively (HR 1.9, $p = 0.05$), predominantly due to excess GVHD deaths in the MMF group. In advanced disease, OS was better with CSA/MMF; 20% and 12%, respectively (HR 0.5, $p = 0.04$), predominantly due to less relapses. In conclusion, GVHD prevention regimen has major impact on outcome after MUD SCT. CSA/MMF is a less toxic regimen and allows prompt engraftment, but is less effective in preventing GVHD. In early stage disease, outcome is dominated by NRM and more effective GVHD prevention regimen such as CSA/MTX is needed. In advanced disease both toxicity and relapse increase. A less intensive regimen, that also better preserves GVL, such as CSA/MMF, may be associated with better outcome. The GVHD prevention regimen selected may need to be tailored to pt and disease characteristics.

489

RITUXIMAB TREATMENT IN CHRONIC GVHD: CLINICAL EFFICACY ASSOCIATES WITH RESTORING A PHYSIOLOGICAL B-CELL BALANCE

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Introduction: Chronic graft-versus-host disease (cGVHD) is the major long term complication of allogeneic stem cell transplantation (allo-SCT). Although the contribution of donor T cells in the development of GVHD is beyond doubt, the role of B cells is less defined. Promising results of B-cell depleting therapy with rituximab (RTX), suggest that B cells do contribute to cGVHD. However, there is no consensus on the exact mechanism by which B cells can cause allo-reactivity.

Objective: In order to elucidate efficacy in a prospective clinical trial and elucidate the potential role of B cells in allo-reactivity and how B-cell depletion results in amelioration of steroid refractory extensive cGVHD, T- and B-cell subsets were studied before and after RTX treatment.

Methods: In a prospective phase I/II study 20 patients with steroid-refractory extensive cGVHD were treated with RTX (4 x 375 mg/m^2). Clinical responses were monitored monthly for 1 year according to the NIH criteria. T-cell and B-cell subsets and phenotypes were analyzed before and after treatment in responders and non-responders and compared to time matched no GVHD controls (no GVHD) and healthy donors (HD) by FACS analysis. (EudraCT 2008-004125-42).

Results: A total of 80 RTX infusions were administered to 20 patients. Toxicity was limited to one infectious event and one allergic reaction. Median follow-up was 6.5 months (range 1-13). Overall response rate was 62%. Best clinical responses were observed in patients with deep sclerosis of the skin (80%). Before treatment absolute numbers of B cells were increased in responders compared to non-responders, no GVHD and HD. Moreover, selectively in responders also the balance within B-cell subsets was disturbed. One year after RTX treatment B-cells could be detected and responders had a restored physiological B-cell phenotype.

Conclusion: RTX treatment is a feasible and effective treatment in patients with steroid refractory cGVHD. Sclerotic lesions of the skin are most susceptible to treatment. In responders the increase in B cells before treatment and the disturbed phenotype suggest that a disbalance in B cells is involved in inducing cGVHD. This allows to speculate that cGVHD is heterogenous and can be either B-cell or rather T-cell mediated. Analyzing B-cell numbers and phenotype in patients with cGVHD might be useful in upfront identification of patients which will benefit from RTX treatment and include patients which will not in alternative clinical trials.

490

STANDARDIZED EARLY INITIATION OF A "HIGH DOSE STEROID (HDS)" TREATMENT PROTOCOL FOR ALLO-IMMUNE LUNG SYNDROMES IS ASSOCIATED WITH IMPROVED SURVIVAL DESPITE THE PRESENCE OF HIGH VIRAL LOADS OF "COMMON COLD VIRUSES"

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Background: Alloimmune lung syndromes (allo-LS), including Idiopathic Pneumonia Syndrome (IPS) and Bronchiolitis Obliterans (BOS), are severe life-treatening complications after HSCT. We recently found that a "common cold" respiratory viral (RV) infection early after HSCT is an important predictor for the development of allo-LS and that prolonged administration of immunosuppression, in patients with a RV infection, because of aGVHD, paradoxically had a protective effect on the development of allo-LS (BBMT 2010). We therefore hypothesised that despite presence of a RV, alloLD should be treated with 'high dose steroids (HDS)". We prospectively studied the outcomes of the treatment of allo-immune lungsyndromes (alloLS) with a standard HDS-protocol" and compared this with our historical cohort.

Methods: All patients transplanted between January 2004 and July 2010, within our pediatric transplant program were included. All patients were tested for the presence of a RV using qPCR prior to HSCT and subsequently weekly till discharge. After discharge only when having symptoms. Allo-LS (IPS or BOS) was diagnosed according international criteria, excluding infection (except presence of a RV). In 2006 the HDS treatment protocol was introduced when allo-LS was suspected: MP-pulse (10mg/kg: 3 days), followed by 2mg/kg/d prednisone, tapered 25% per week till 0.5mg/kg. After 4 weeks the MP-pulse was repeated, followed by 0.5mg/kg/d for at least 1 months. In case there was still suspicion of disease the MP-pulse was repeated (max. 6times). Ciclosporine tough levels were maintained between 150 and 250 ug/L. In the old treatment guidelines we were reluctant giving HDS because of the presence of a RV.

Results: 182 patients were included of whom 38 (21%; 15% IPS and 6%BO) developed an allo-LS. 35/38 of the patients with an alloLS had a proven RV. Follow up: 36mths (1–76 mths). The overall survival was 63% (73% without alloLS, 43% with alloLS). Cause of death in the alloLS group was 90% TRM and 10% relapse. 10 pts were treated according to old treatment guidelines, while 28 were included in the HDS protocol. The probability of OS was 20% for the "old treatment group" while 54% of the patients within the HDS group survived ($p = 0.041$). The viral load of the RV, expressed as CT-values, remained stable (median value 20: range 17 – 24) during the treatment with HDS.

In summary: Early initiation of HDS in alloLS improved survival, despite the presence of a RV (infection).

491

DISTINCT OLIGOCLONAL T CELLS ARE ASSOCIATED WITH GVHD OR GVHD-FREE RESPONSES IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES FOLLOWING STEM CELL TRANSPLANTATION

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Graft-versus-host disease (GVHD) and graft-versus-tumor (GVT) effects observed following allogeneic stem cell transplantation may be associated with unique clonal T cell expansion. To

better elucidate this, our group is conducting a prospective study that evaluates the T cell receptor (TcR) Vβ repertoire from patients who are undergoing either matched related (MRD) or unrelated (URD) donor allogeneic hematopoietic stem cell transplantation (HSCT) for treatment of hematologic malignancies. These patients received conditioning therapy with a regimen combining rabbit anti-thymocyte globulin (Thymoglobulin®, Genzyme, Cambridge, MA) and 450 cGy total body irradiation. We used a novel two-tier analysis of TcR Vβ mRNA expression in order to identify GVHD and/or GVT-associated T cell clones: i) normalization of the TcR Vβ mRNA to CD3 mRNA rather than to housekeeping genes using real-time RT-PCR which increases sensitivity of the assay for detecting minimal circulating T cell clones of interest that are activated; ii) comparative analysis of the recipients' TcR Vβ over those of the donors' at the time of GVHD or GVT effects up to 1 year post-HSCT. We then performed spectratyping analysis to confirm clonality of the relevant T cell clones. We have been able to identify T cell populations in the HSCT recipients at 90 days to 1 year following transplant, by demonstrating that distinct TcR Vβ transcripts were significantly increased relative to the steady state expression in the donor, in patients without GVHD or relapse vs. patients with GVHD. In the three GVHD-free, relapse-free patients, we found an increased expression of Vβ 9 in all patients as well as an increased expression of Vβ 16 and Vβ5/Vβ 24 in two and one patient, respectively. In 6 patients with GVHD, we observed increased expressions of Vβ4 and Vβ11 in four patients and increased Vβ23 in three patients. Spectratyping analyses of each of these TcR Vβ families showed distinct skewed, mono- or oligoclonal populations, in patients with GVHD or GVHD- and relapse-free states. The identification of these unique T cell clones in patients who have developed GVHD or GVHD-free responses after allogeneic HSCT suggests that they may be driven by antigen stimulation uniquely associated with GVHD or GVT. Identification of GVHD-associated T cell clones may offer aan opportunity for targeted therapy of GVHD rather than using conventional immunosuppressive drugs.

492

SIROLIMUS, TACROLIMUS, AND RABBIT ATG (R-ATG) AS GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN PATIENTS UNDERGOING UNRELATED DONOR PERIPHERAL BLOOD HEMATOPOIETIC CELL TRANSPLANT (HCT): EXTENDED FOLLOW UP AND UPDATED ANALYSIS

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The combined use of Sirolimus (SIR) and Tacrolimus (FK) +/− low-dose methotrexate (MTX) recently showed a promising result in preventing acute GVHD after unrelated donor HCT (Antin et al. Blood 2003) although a significant number of patients still experienced chronic GVHD. In an attempt to further improve the outcome, we have evaluated a novel combination of Tacrolimus, Sirolimus and r-ATG (4.5 mg/kg) +/− Methotrexate (for patients with a mismatched donor). We have previously presented the early results of this combination. In this report we extended the follow-up period and updated analysis focused on 49 patients who received PBSCT (See Table 1 for patient demographics).

Results: Engraftment rate was 91.8% (n = 45) with the median neutrophil engaftment at 15 days (range: 10-39). Seventeen patients (38% of 45 engrafted) developed grade II-IV acute GVHD (grade III = 3, IV = 0). Chronic GVHD developed in 23 of 38 evaluable patients (60%, limited n = 4, extensive n = 19). We observed TTP/HUS in 10 patients (20%) and one case of VOD. Eighteen (42%) of 43 patients at risk (Recipient +ve or Donor +ve) developed CMV reactivation, while 10 patients developed EBV reactivation (19.6%). After a median follow up of 24 months (range: 6-48) for 33 surviving patients, the **1-year OS, DFS, and relapse rate** were **75.4%** (95%CI: 64.8-83.2), **73.4%** (63.0-81.3), and **8.2%** (3.2-21.0), respectively. Non-relapse mortality (NRM) was 16.3% (8.7-30.8) at 100 day and 18.4% (10.2-33.2) at 1 year. There were no significant differences in the outcomes according to conditioning regimens, although there was a trend for lower NRM with Flu/Mel (9.7% vs. 27.8% at 1 year, p = 0.06).

In summary the combination of FK/SIR with r-ATG +/− MTX may improve the GVHD and survival outcome in this high-risk population. However, we observed graft failure in 4 patients, which appeared to be a higher rate than our historic data of approximately 2% in the setting of unrelated donor HCT.

Table 1. Patient Demographics

Variable	All Patients N=49 (100%)
Patient Gender	
Female	28 (57.1)
Male	21 (42.9)
Median Age at transplant	57 (19-71)
Disease / Disease status	
ALL/CR1 and CR2	9
ALL/Induction failure	1
AML/CR1 and CR2	12
AML/Relapse and Induction failure	7
CML/CP1 and CP2	2
CML/ AP and BC	2
NHL	5
CLL	1
MDS	8
MPN	2
Conditioning Regimen	
Reduced Intensity Fludarabine/ Melphalan	31
FTBI/Cytoxan	7
FTBI/VP-1611	11
HLA Match	
10 out of 10	26
others	23

ALL; Acute Lymphoblastic Leukemia, CR; Complete Remission, CP; Chronic Phase, AP;Accelrated Phase, BC;Blast Crisis, NHL; Non-Hodgkin Lymphoma, CLL;Chronic lymphocytic leukemia, MDS;Myelodysplastic Syndromes, MPN; Myeloproliferative Neoplasm

493

NRF2 REGULATES ALLOREACTIVE T CELL FUNCTION DURING GVHD

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The activation and regulation of donor alloreactive T cells is critical for the development of graft-versus-host disease (GVHD) as well as the regulation of graft-versus-tumor (GVT) activity. To better understand transcriptional orchestration of alloreactive T cells, we employed Finding Informative Regulatory Elements (FIRE) approach, recently published by Elemento et al, to investigate gene expression data in donor T cells in murine syngeneic (syn-) and allogeneic bone marrow transplant (allo-BMT) models. We discovered the presence and absence of the motif CCGGAAG, recognized by the transcription factor Nuclear Factor Erythroid 2-Like Factor 2 (Nrf-2), correlated to a set of genes that were slightly upregulated in syn- and downregulated in allo-donor T cells. We hypothesized that Nrf-2 serves as a master regulator of genes in alloreactive donor T cells involved in the GVHD setting.

We first examined the level of expression of Nrf-2 in donor T cells in murine syn- and allo-BMT models. We found that the expression of Nrf-2 was significantly reduced only in the regulatory T cell population (CD4+CD25+Foxp3+) in allo-BMT ($p = 0.0004$). We next questioned the significance of Nrf-2 in alloreactive T cells. Adoptive transfer of Nrf2-/- donor T cells leads to less GVHD morbidity, as characterized by weight loss ($p < 0.0001$) and better survival ($p = 0.0030$), compared to Nrf-2+/+ donor T cells. Interestingly, loss of functional Nrf-2 in donor T cells did not perturb their GVT activity at the studied dose (A20, 0.25x106 per host). We demonstrated that CFSE labeled Nrf2-/- donor T cells have intact proliferation and activation (as determined by the upregulation of CD25) upon stimulation by alloantigen. Lastly, we investigated the trafficking ability of Nrf2-/- donor T cells to gut-associated lymphoid tissues. Although not statistically significant, there were less Nrf2-/- donor T cells infiltrating the mesenteric lymph nodes (MLNs) compared to Nrf2+/+